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(54) Title: USE OF SUBSTANCE P ANTAGONISTS IN THE TREATMENT OF THE ADENOCARCINOMAS

(57) Abstract: It is described the use of antagonists of neuro kynin receptors (NK-1; substance P receptors) in order to establish a new drug effective in the treatment of adenocarcinomas, such antagonists being one or more substances selected among those ones having the following features: pA_2>6.0 both in human and in murine tissues, etherocyclic non peptidergic structures, antiangiogenic effects experimentally demonstrated onto the genito-urinary tract tumors induced via orthotopic drafts of human tumoral cells either in the genito-urinary apparatus of either immunodeficient rats or mice, decrease of the tumoral mass on tumors of the genito-urinary tract induced by orthotopic drafts of tumoral cells onto tissues of the genito-urinary tract of either immunodeficient rats or mice.

USE OF SUBSTANCE P ANTAGONISTS IN THE TREATMENT OF THE ADENOCARCINOMAS

Field of invention

The present invention refers to the use of substance P antagonists for the treatment of adenocarcinomas, particularly genito-urinary-tract neoplasms, more particularly prostatic carcinoma.

State of the art

Malignant neoplasms, originating from epithelial cells are named carcinomas. A peculiar type of carcinoma, of glandular origin, is the adenocarcinomas.

Since a correlation between tumors and angiogenesis has been hypothesized, a possible, effective strategy against cancer disease is a pharmacological treatment with angiogenesis inhibitors.

Angiogenesis, i.e. the formation and development of new capillary vessels, occurs in different physiologic conditions, such as embryonal development. On the other hand, intense angiogenesis occurs in several pathologic conditions, such as synovial rheumatoid hypertrophy, atherosclerosis, proliferative retinopathy and solid tumors. With reference to solid tumors, the interest in the neovascularisation has been raised from the evidence that tumors cannot growth or metastasize without new vessels and/or growth factors. Solid tumors cannot grow beyond 1-2 mm^3 without neovascularation, which furnishes feeding to tumors. Therefore, experiments have been carried out in order to quantify neovascularation in order to try to evaluate the tumor growth at different stages [Tosan A., Fregene et al, Anticancer Research 13: 2377-2382 (1993); 13: Brigitte V. Offersen et al., APMIS 106: 463-469 (1998)]. Consequently, it has been hypothesized that tumor growth could be prevented by neovascularization blockage.

Experimental evidences have recently outlined that substance P (SP) plays a role in angiogenesis stimulation:

Daily administration of substance P causes intense neovascularization in a rat sponge model of angiogenesis [T.-P.D. Fan et al, Br. J. Pharmacol. 110: 43-49 (1993)];

The angiogenic response towards SP can be blocked by using selective antagonists for NK-1 receptors for tachykinines [T.-P.D. Fan et al, Br. J. Pharmacol. 110: 43-49 (1993)];

The angiogenic activity of SP can be counteracted by administration of either peptide or non peptide antagonists [D. Regoli et al., Pharmacol. Rev., Vol. 64, No. 4 551-559 (1994)].

Such experimental results have not yet led to identify any drug which could be successfully employed in the treatment of the adenocarcinomas, particularly the prostatic carcinoma. As a matter of fact, Fan et al. states about future therapeutic applications in the above mentioned mechanism.

Moreover, NK-1 antagonists show a great variability in their molecular structure (Regoli et al, 1994), therefore being very difficult to predict potential activity based upon their structure-activity relationship. Also, insofar, no data have currently shown that the antagonistic activity and selectivity towards the human subtype NK-1 receptor could be of interest in the therapy of adenocarcinoma, particularly of the prostatic adenocarcinoma. In relation to the current state of the art regarding cancer therapy, none of the tested substances seems to be effective in cancer therapy. Therefore for the substances mentioned in Regoli it cannot be inferred any specific activity against cancer.

With the present invention, we aim at inhibiting, via administration of inhibitors of angiogenesis and particularly by using of antagonists of NK-1 tachykinergic receptor, solid tumor growth specifically localized to the genito-urinary tract.

This innovative methodology either substitutes or integrates current therapies against cancer, such as surgery, chemiotherapy and radiotherapy.

Summary of the invention

It is an object of the present invention the use of NK-1 receptor antagonists in the treatment of the adenocarcinomas, particularly in the treatment of the carcinomas of the genito-urinary tract and more particularly in the treatment of the prostatic adenocarcinoma.

Another object of the invention is the use of substance P antagonists in the treatment of the adenocarcinomas, particularly in the treatment of the carcinomas

of the genito-urinary tract and more particularly in the treatment of the prostatic adenocarcinoma.

Further objects of the invention will be evident by the following detailed description of the invention.

Detailed description of the invention

The present invention refers to the use of substance P receptor antagonists in the treatment of the solid tumors, particularly the ones originating from the genito-urinary tract. Selected antagonists according to the present invention are listed in Tab. 1 and are characterised by the following features:

1) pA_2 >6.0 both in murine and human tissues, pA_2 being the concentration of the drug at which it is half-maximally effective, and the pA_2 is directly related to the affinity of the ligand to the receptor,

2) Non peptidic etherocyclic structure

3) Antiangiogenic effects experimentally evaluated onto tumors of the genito-urinary tract induced via orthotopic grafts of tumoral human cells on tissues of the genito-urinary tract of rats and/or immunodeficient mice. Orthotopic graft is intended to be a graft of cells in the host, via direct injection of cells.

4) Reduction of the tumoral mass experimentally demonstrated onto tumors of the genito-urinary tract induced via orthotopic grafts of tumoral human cells on tissues of the genito-urinary tract of rats and/or immunodeficient mice.

The following substances are considered by the authors effective against adenocarcinomas, specifically against the adenocarcinomas originating from the genito-urinary tract.

Table 1

Compound	pA_2 human	pA_2 rat	References
FK 888	9.1	6.0	Fujii et al., Br. J. Pharm. 107:785, 1992
CP 96345	9.5	6.8	Srider et al., Science 251:435, 1991
CP 99994	8.9	6.1	Desai et al., J. Med. Chem. 35: 4911, 1992
SR 140333	9.8	7.4	Edmonts et al., Eur. J. Pharm. 250:403, 1993
CGP 47899	>0	>6.0	Shilling et al., Pers. Med. Chem. 207, 1993

RP 67580	7.2	8.2	Garret et al., PNAS 88:10208, 1991
MEN 11149	>6.0	>6.0	Cirillo et al., Eur. J. Pharm. 341:201, 1998
MEN 11467	>6.0	>6.0	Evangelista et al., XXIX Nat. Congr. of the Ital. Pharmacological Soc., Florence 20-23.06.1999
GR 205171	>6.0	>6.0	Gardner et al., Regul Pep. 65:45, 1996
L-703,606			Cascieri et al., Mol. Pharmacol. 42, 458, 1992

The above mentioned substances may be used to prepare drugs in combination with known adjuvants. Sinergistically, they can be combined with substances listed in table 2. As a matter of fact, the combination of one or more substances listed in table 1 with one or more substances in table 2 (or corresponding derivatives of the substances listed in table 2, derivatives known to the man skilled in the art) provides a therapeutic positive response higher than 10%, as stated in points 3-4 of the pharmacological characteristics of the NK-1 antagonists.

Table 2

FLUTAMIDE (Eulexin by Schering; Drogenil by Essex). Preferred dose range: 15-1500mg/day

LEUPROLIDE ACETATE (Enantone by Takeda). Preferred dose range: 0.1-10mg/month

GOSERELIN (Zoladex by Zeneca). Preferred d ose range: 0.1-10mg/28 days

AMINOGLUTETHIMIDE (Orimeten by Ciba-Geigy). Preferred dose range: 30-3000mg/day

KETOKONAZOLE (Zinoral by Janssen). Preferred dose range: 10-1000mg/day

DOXORUBICINA (Adriblastina by Pharmacia & Upjohn). Preferred dose range: 2-100mg/day

TAXOL Preferred dose range: 2-100mg/day

Route of administration is based upon the specific characteristics of the compounds and implies the endovenous, intrabladder, intraperitoneal, intramuscular, subcutaneous and oral administration.

Adjuvants are selected among those commonly used in pharmacotherapy, such as: methyl-p-hydroxybenzoate, latex and saline solution.

Administration of substances listed in table 1, possibly in combination with substances listed in Table 2, is able to either reduce or reverse tumor growth via inhibition of angiogenesis and tumoral mass.

Moreover, use of antagonists of substance P is endowed of the following advantages:

Antiemetic effect, opposite to chemiotherapic drugs which show marked emetic effects

Antidepressive effect, which is a very important psychotherapic effect in cancer affected patients.

The following examples should be considered as illustrative of the present invention an not limitative of the scope of the invention itself.

Materials and Methods

Animals

For this studies has been used male athymic nude mices (Harlan). The mices were housed in laminar flow cabinet under pathogenic free conditions and used at 4-5 weeks of age.

Orthotopic implantation

The PC-3 human prostatic cancer cell lines (ECACC) were mantained in Minimum essential medium (GIBCO BRL) For *in vivo* studies, tumor cells in exponential growth phase were harvested by a 120 seconds treatment with tripsyn in 0.02 % EDTA. After that the cells were resuspended in saline solution. 20 µl (10^5 cells) of saline solution was inoculated in nude mice prostate. The tumors were taken at different times (three up to 180 days) for analysis.

Treatments

Animals were divided in groups (10 animals each one) and treated with different solutions:

- physiological solution, - Sustance P (1 ng up to 1 mg), - L-703,606 (1 ng up to 1 mg), - L-703,606 + Leuprolide Acetate (using dosages reported in table 1 and 2).

The treatments were made at different time (one at day until one at week). For different periods of time (three days up to 180 days).

Immunohystochemistry

Frozen sections of tumors have been fixed in Acetone, acetone/chloroform, acetone.

The presence of new blood vessels has been highlighted using the Rat anti-Mouse CD31 antibody, a Goat anti-Rat POX as a secondary antibody and DAB as a chromogen substrate. For quantification of blood vessels an image analysis system was employed.

Cancerogenicity

The animals were maitained under observation for three up to 180 days. Autoptic examinations were performed in all animals and the tumoral mass weighed.

Results

Vascular density quantification

The quantitative data obtained on blood vessels density have shown a statistically significant difference ($p<0.05$) amongst different treatments. SP treated mice group showed a statistically significant increase of CD 31 values compared to control groups ($p< 0.05$).

Moreover, a statistically significant reduction ($p<0.05$) of CD 31 values has been outlined in mice treated with L-703,606 compared either to control groups or SP treated mice. The inhibitory angiogenic effect mediated by L-703,606 has been shown in all groups treated with this compound. A statistically significant reduction of CD 31 values has been shown with L-703,606 + Leuprolide Acetate ($p<0.05$) compared to L-703,606 groups (>10%).

Tumoral mass weight

Tumoral mass weight has been evaluated in all experimental groups, throughout treatment days (from 3 up to 180 days). Results have shown a statistically significant reduction ($p<0.05$) of tumoral mass weight in mice treated with L-703,606, compared to either control groups or SP treated mice.

Moreover, combination of L-703,606 with Leuprolide Acetate allows to reveal a further tumoral mass reduction, (>10%).

Correlation between tumoral mass weight and type of treatment is proportionally related to treatment time-course.

CLAIMS

1) Use of antagonists of NK-1 receptors to prepare a medicament for treating adenocarcinomas, such antagonists being one o more compounds and corresponding mixtures selected among the compounds having the following characteristics:

- $pA_2 > 6.0$ in human and murine tissue;
- non peptidic etherocyclic structure;
- antiangiogenetic effects experimentally demonstrated in uro-genital tumors, induced by orthotopic implantation of human tumoral cells in rat and mice urogenital tissues;
- reduction of tumoral mass in uro-genital tumors induced by orthotopic implantation of human tumoral cells in rat and mice urogenital tissues.

2) Use according to claim 1 wherein the compounds are selected among: FK 888, CP 96345, CP 99994, SR 140333, CGP 47899, RP 67580, MEN 11149, MEN 11467, GR 205171, L-703,606 and corresponding derivatives and corresponding mixtures.

3) Use according to claims 1 and 2 wherein the compound is combined with at least one compound selected among: FLUTAMIDE, LEUPROLIDE, GOSERELIN, AMINOGLUTETHIMIDE, KETOKONAZOLE, DOXORUBICINA, TAXOL and corresponding derivatives and corresponding mixtures.

4) Use according to claim 3 wherein each of the compounds is administered at the following dosage: FLUTAMIDE 15-1500mg/day, LEUPROLIDE ACETATE 0.1-10mg/month, GOSERELIN 0.1-10mg/28 days, 30-3000mg/day, 10-1000mg/day, DOXORUBICINA 2-100mg/day, TAXOL 2-100mg/day.

5) Use according to claims 1-4 wherein the compounds are administered via endovenous, intrabladder, intraperitoneal, intramuscular, subcutaneous and oral route.

6) Use according to claims 1-5 wherein the compounds are mixed with adjuvants selected in the group consisting of: metil-p-hydroxybenzoate, latex, saline solution and corresponding mixtures.

7) Use according to claims 1-6 to prepare a medicament for treating uro-genital carcinomas.

8) Use according to claims 1-6 to prepare a medicament for treating prostatic adenocarcinomas.